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Thyroid Stimulating Hormone (TSH) and Thyroxine Hormone (T4) Role in Thyroid Detriment on Hypothyroidsm Animal Models

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Abstract: Hypothyroidism is a condition where thyroid gland encounter on degradation. It causes many complication impacts especially on hormone metabolism. This previous study had developed the preparation of hypothyroidism animal model by injected of goat Thyroglobulin (cTg) through detriment of tyroid gland, as well as other impact to rat *(Rattus norvegicus)* as experimental animal model. This study was followed by optimizing the preparation also the exhaustively observation of rat animal model. Based on previous worked, preparation of animal model were conducted by intravenous injected of 200 µg/mL of goat thyroglobulin (cTg) with Complete Freund's Adjuvant (CFA) at 1:1 ratio continued with booster on Incomplete Freund's Adjuvant (IFA) at same ratio. Then levelthyroid stimulating hormone (TSH)and thyroxine hormone (T4) were measured then analyzed with the alteration on thyroid gland to evaluate the development of the disease. This research showed, injection of cTg could lead to decrease of T4 levels and increase TSH levels. Meanwhile the thyroid gland which were had been injected with cTg showed the different size, structure also the mononuclear cells infiltration compare to control group. The binding of cTg and thyroid follicle caused damage and dysfunction of thyroid gland. These findings showed the hypothyroidism condition can alter other hormone produced gland which lead to development of diseases.
Keywords: hypothyroidism, goat thyroglobulin, throxine, thyroid stimulating hormone.

Introduction

Autoimmune thyroiditis (AITD) is an autoimmune disease in which the thyroid specific organ. AITD can occur due to the induction of rat thyroglobulin (mTg), cattle thyroglobulin (bTg) and swine thyroglobulin (pTg) [1]. This disease can cause damage to the thyroid gland, causing thyroid hypertrophy and increase metabolism followed by stimulation of the thyroid[2]. Thyroid it is autoimmune disease can be detected by the formation of auto antibodies TPO and thyroglobulin. One type of disease is hypothyroidsm[3]. Hypothyroid condition leads to dysfunction of hormone metabolism.

Specific markers Hypothyroidsm are Thyroid peroxide as eautoantibodies (TPO) and thyroglobulin (Tg). The existence of the set womolecular markersis crucialin knowing the development of the thyroid gland

damage due to autoimmune disease processes. Tyroglobulinis a660k Daglycoprotein as a storage place and producing thyroid hormone with support of iodine. WhileTyrosine peroxidase is an enzyme that works to release iodineis add edto the tyrosineresiduesin thyroglobulin. It lead to produce thyroxine (T4) and triiodhotyronin (T3) [4]. Our previous worked had demonstrated the preparation of hypothyroidsm animal model using goat Thyroglobulin (cTg) with dose of 100 µg/mL and 200 µg/mL on four weeks. This recent work we had optimized the preparation of this animal models using 200 µg/mL with extended observation on various impact [5].

Material and Methods

Animal Model Preparation

This study used female *Rattus norvegicus* Wistar strain obtained from the Animal Model Unit Development (UPHP) UGM Yogyakarta with 8-12 weeks of age, body weight between 100-150 g and approved by Brawijaya University Research Ethics Committee No. 140-KEP-UB. Animals were divided into two groups; (A) control group without cTg induction; and (B) rat with cTg induction dose of 200 µg/mL emulsified with complete Freund's adjuvant (CFA) and boosted with same dose and incomplete Freund's adjuvant (IFA) (1:1).

Thyroglobulin Isolation

Goat tyroglobulin(cTg) isolation procedures were conducted by mixing 1 g of goat's thyroid, 5 mL of PBST-PMSF in a cold mortar. Homogenates mixed by vortexing, sonified and centrifuged. Supernatant then was transferred and added with absolute ethanol. After a night, ethanol was discharged, resulted of cTg proteins as pellet obtained were added with TrisHCl (1:1).Goat tyroglobulin(cTg) protein levels were measured using the biuret test. cTg protein levels obtained were 570 µg/µl. The amount of protein used was 200 µg/mL.

cTg Injection

Injection of cTg was conducted by intravenous injection withComplete Freund's Adjuvant (CFA) at 1:1 ratio on first day treatment. Booster was done at 14^{th}days and 28^{th} days with cTg and Incomplete Freund's Adjuvant(IFA) at 1:1 ratio[1,6] with some modifications.

Blood Serum Isolation

Blood was taken from heart after rat scarification. Blood was then put on vacutainer tubes and incubated at room temperature for 3 h to serum components. Serum then was purified wih 50% Saturated ammonium sulphate (SAS) stored in 1.5 ml tube [7]. Blood sampling were collected in the first day and after 8 weeks of treatments.

Measurement of TSH Hormone and Thyroxine (T4) hormone levels

Hormone levels of Thyroid Stimulating Hormone (TSH) and tetraiodotironin/ thyroxine (T4) from serum were measured using Rodent TSH and T4 ELISA Test Kit (Endocrintech). Standard and sample solution of 50 µl was put in well then add serum that has been diluted as much as 50µl. In each well was then added 100µl TSH or T4 HRP-conjugate reagent. Microplates then were incubated at 37°C for 60 min then washed with washing buffer. In each well was then added with TMB reagent then covered with aluminum foil and incubated at 37°C for 20 min. The last, each wells were added with 50µl stop solution (2N HCl) in and then measured using ELISA Reader.

Dissection and Thyroid Isolation

Dissection was performed in all groups at week 8. Rats were sacrificed with dislocation followed by blood collection through the heart and thyroid isolation on each animal group. Thyroid organ was cleaned with 0.9% Na-Physthen put in 10% paraformaldehyde chamber for histopathology slides production.

Histopathology Slides Production

The process of histopathology preparations consisted of fixation, dehydration and infiltration, purification, paraffin infiltration, embedding, sectioning, pasting on glass object then staining using hematoxylin-eosin [7].

Result and Discussions

Thyroid stimulating hormone (TSH) produced by the anterior pituitary and serves to stimulate the production of thyroid hormones. In normal conditions, TSH will stimulate the thyroid gland to produce thyroid hormones which secreted next to metabolism process and inhibit TSH through a negative feedback mechanism when thyroid hormone levels produced are sufficient [8]. On this research, hypothyroidism conditions were conducted by injecting of caprine Thyroglobulin (cTg) lead to the increases of TSH (Table. 1). Statistical analysis showed that it was significantly difference between groups. cTg injection could increase 124% TSH level compare to normal condition.

Table 1.Thyroid Stimulating Hormone (TSH) and Thyroxine (T4) Hormone

Groups	TSH Hormone Levels (ng/ml)	T4 Hormone Levels (ng/ml)
Normal	0.139 ± 0.011	1.722 ± 0.023
cTg injection	0.312 ± 0.021	0.813 ± 0.018

The damaged and impaired function of thyroid gland caused interference of thyroid hormone production. Ferguson [9], explained that thyroid hormone secretion is influenced by the condition of thyroid gland with two physiological mechanisms, namely the autoregulation mechanism based on the availability of iodine content and feedback mechanisms of the hypothalamus and anterior pituitary. The impaired function of of thyroid gland in this study was caused by the immune response of rat animal models against cTg injection. cTg will be recognized as antigen by immune system and cause the formation of autoantibodies to thyroglobulin which produced by thyroid gland. Thyroglobulin autoantibodies formation will cause the damaging of thyroid gland due to the similarity of physical, biochemical and molecular structure in some species. Besides, the bulks containing of thyroglobulin in thyroid gland, resulting the formation of autoantibodies against thyroglobulin will lead to thyroid gland damaged [6].

In other hand, the damaging of thyroid gland will decrease the thyroxinehormone (T4)levels in blood circulation (Table1.). Measurement of thyroxine hormone (T4) showed differences between groups due to induction of 200 µg/mLcTg.Piechotta, *et al.* [10], explained that the decreasing levels of T4 level showed hypothyroidism condition. In this condition, thyroid gland was unable to produce enough thyroid hormone to maintain the stability of organs. This work was accordance with previous study [11]. One of effects of hypothyroidism is disruption of hypothalamic-pituitary-lane thyroid, which caused no negative feedback on anterior pituitary, so TSH will be produced continuously.The increasing levels of T4 showed the progressive of Hypohyroidism[12].

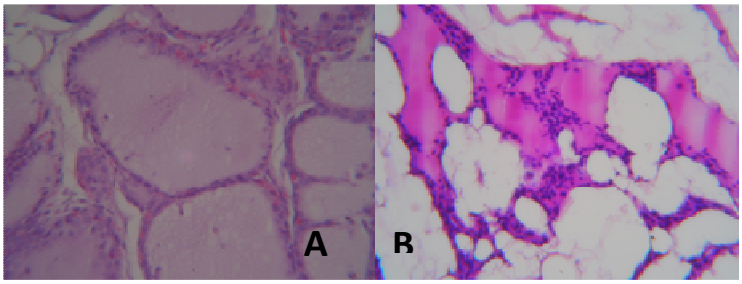

Figure 1.Thyroid Gland appearance on (A).normal, and (B) Hypothyroidsm rats; HE staining (400x)

Histopathological observation of thyroid gland of treatment groups is showed as follows: Fig. 1 showed that in normal condition (Fig. 1A), thyroid gland wa scomposed of irregular follicle wallandlumen containing colloidbrightly colored. Thiscondition was differentin the thyroid gland of hypothyroidism rats which show edtissue damage (Fig. 1B). It showed the composition of thyroide pithelialcells was irregularand did not surround by thethyroidfollicles. It also showed mononuclearcellinfiltrationanddestructionof thyroidfolliclesdue to injection of cTg. These results were pretty much the same withother reports [6]which used rat thyroglobulin (mTg), and [13] which used swine thyroglobulin (pTg). It was indicated that the alteration of shape and follicle thyroid structure lead to damaging of thyroidtissues. It was also occurring of infiltration of mononuclear cells.

Conclusions

Hypothyroidsm induced with cTg injection lead to alteration of main hormone such as thyroid stimulating hormone (TSH) and Thyroxine (T4). It had been produced the increasing of TSH level and decreasing of T4 compare to normal condition. It also showed the detriment of thyroid gland.

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